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THE ETIOLOGY OF EPIDEMIC POLIOMYELITIS

PLATES 6-8

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A somewhat peculiar diplococcus or streptococcus has been found in the spinal fluid, and in the brain and cord of cases of poliomyelitis by various observers. In some instances injections in animals gave suggestive, although inconclusive results indicating the causal relationship of this organism. Other workers were unable to obtain corroborative evidence. Efforts to demonstrate bacteria in sections proved unsuccessful. The discovery that typical poliomyelitis can be produced in the monkey with so-called virus and filtrates of virus,[4, 9, 10] and the successful inoculation of monkeys by Flexner and Noguchi with the small "globoid" organism which they cultivated from the central nervous system in poliomyelitis, was considered final in proving that bacteria of ordinary size had no etiologic relation to this disease. The status of the question of the relation of these organisms, particularly streptococci, to poliomyelitis, when we began our studies is tersely stated by Wickman: "Such bacteria must be regarded as having had an accidental and not a causal relation to the malady."

However, since the earlier studies were made, special methods have been developed and bacteria of ordinary size have been isolated from tissues which were considered sterile. The localization of these bacteria in animals frequently corresponded closely to that in the disease from which they were isolated. In view of these results, the older investigations were considered inconclusive as proving that bacteria of ordinary size had no etiologic relationship to poliomyelitis, and a restudy of the bacteriology of poliomyelitis by the use of the newer methods was undertaken.

We wish now to record the details of a study of a series of cases of poliomyelitis which occurred in Rochester, Minnesota, and New York City during the epidemic of 1916, and in Davenport, Iowa, in 1917. The results of the cases occurring in 1916 have been described in a preliminary report[19] in which the following statement was made:

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TABLE 1

RESULTS OF A STUDY OF CASES OF ACUTE EPIDEMIC POLIOMYELITIS

No.	Age in Years	Duration in Days	Clinical Findings	Necropsy Time after Death	Microscopic Changes
707	4⅔	7	Paralysis of muscles of left side of face, neck and deglutition. Death from respiratory failure	Day of death	Hemorrhage, moderate perivascular and disseminated round-cell infiltration, chiefly in anterior horns and in pia over anterior aspect of cord and medulla
712	4	5	Paralysis of lower extremities, right shoulder and neck. Death from respiratory failure	8 hours	Extreme perivascular and diffuse infiltration chiefly of gray matter of cord and medulla. Perivascular infiltration most marked around vessels extending in horizontally from pia. Moderate infiltration of pia especially around blood vessels and in anterior fissure. Hyperplasia and necrosis of lymphoid follicles in tonsils (Fig. 8). Hemorrhages in mucous membrane of stomach. Small areas of necrosis with round-cell accumulations in liver. Viscera normal otherwise.
714	2	15	Paralysis of legs and shoulders. Death from respiratory failure	4 hours	Cord showed extreme dilatation of vessels; hemorrhages marked degeneration of ganglion cells; slight neurophagocytosis, moderate perivascular and diffuse infiltration with round cells. Slight infiltration in ganglia. No lesions of liver, myocardium, pancreas, kidney and spleen. Hemorrhages and leukocytic infiltration of lung. Marked hyperplasia of lymph follicles in tonsils
720	3	3	Paralysis characteristic of upper spinal type	20 hours	Slight perivascular, moderate infiltration of gray matter of cervical cord with destruction of ganglion cells. No change in viscera
721	9/12	6	Paralysis of meningitic type	Day after death	Typical poliomyelitic changes in cord. No noteworthy changes in viscera other than localized hemorrhages and beginning leukocytic infiltration of lungs
722	3	4	Paralysis of upper spinal type	Day of death	Perivascular infiltration most marked around blood vessels running in from pia, circumscribed areas of round-cell infiltration with destruction of ganglion cells in cord. No changes demonstrable in brain. Areas of necrosis of lymph follicles in tonsils. Liver, pancreas and myocardium normal
723	24	6	Paralysis of right deltoid, right and left quadriceps and psoas, muscles of deglutition and diaphragm	2 hours	Slight round-cell infiltration in pia over cerebrum. Areas of necrosis in lymph follicles in tonsil. Hemorrhages in mesenteric gland. No changes in liver, pancreas, myocardium or kidney
724	1½	3	Paralysis of muscles of neck and left side of face. Death from respiratory failure	12 hours	Brain and cord not saved for microscopic examination
725	1½	4	Paralysis of both legs, back, shoulders and intercostal muscles. Death from respiratory failure	22 hours	Typical poliomyelitic lesions in cord and brain
729	3	9	Paralysis of right side of face, muscles of neck, upper extremities and intercostal muscles. Death from respiratory failure	3 hours	Extreme diffuse round-cell infiltration and numerous hemorrhages in gray matter of cervical cord and medulla. Moderate perivascular infiltration and edema, and slight infiltration of pia, of cerebral cortex and spinal ganglia. Congestion of vessels of liver and kidney. Hyperemia and hyperplasia of lymph follicles in mesenteric gland. Otherwise no noteworthy changes in viscera

TABLE 1—*Continued*

RESULTS OF A STUDY OF CASES OF ACUTE EPIDEMIC POLIOMYELITIS

Bacteria in Sections	Results in Cultures
Large and small cocci and diplococci in pia of anterior fissure and anterior aspect of cord, in infiltrated areas in anterior horns of cervical cord and medulla (Fig. 2)	Micrococcus and short chained streptococcus varying greatly in size and shape from cord. Diphtheroid-like bacilli in old cultures. Streptococcus from brain and cord after 3 and 15 months in 50% glycerin
Large and small diplococci at least 40 in all, in infiltrated area anterior horns (Fig. 3), ganglia, pia, edematous dura and mesenteric lymph glands. None found in kidneys, liver, lungs, myocardium or sciatic nerve. Many similar organisms found in tonsils (Figs. 9 and 10)	Extremely pleomorphic coccus singly in twos and short chains from cord. Spleen, kidney and liver sterile. Streptococcus and colon bacillus from lumbar cord
Large and small diplococci in areas of hemorrhage and infiltration in anterior horns, in ganglia, in smears from anterior horn and contact films from cerebrum (Fig. 5). Many similar organisms in tonsils and diplococci in hemorrhagic area in lungs. No bacteria in liver, kidney, spleen, myocardium and pancreas	Streptococcus from edematous fluid surrounding dura, from cerebral fluid, brain, lumbar cord and from brain after being dried from 6 to 11 months respectively. Kidney sterile. Streptococcus in pure culture from brain and cord after in 50% glycerin 3, 8, 15, and 16 months
Diplococci in anterior horn of cervical cord and intervertebral ganglia. No bacteria in liver, lung and pancreas	Short chained diplococcus from cervical cord and brain
A few cocci and diplococci in peripheral zone of round-cell infiltration in anterior horn. Many similar organisms in tonsils. Many diplococci in hemorrhagic areas in lungs. No bacteria in liver and kidney	Short chained streptococcus and large and small coccus from cervical cord and brain. Hemolytic streptococcus and colon bacillus from cord
A few large and medium-sized diplococci in peripheral zone of infiltrated areas in gray matter of medulla and cervical cord. A few cocci and diplococci in hemorrhagic areas in mesentery gland. Enormous numbers of same organism in necrotic lymph follicles of tonsils together with fusiform bacilli in crypts of tonsil. No bacteria in brain, liver, pancreas, myocardium	Streptococcus and large and small coccus forms in pure culture from material obtained with pipet from brain and cord, and streptococcus and a few staphylococci from pieces of cord emulsified in mortar, and from mesenteric gland
No bacteria demonstrable in brain. Large number of large and small cocci and diplococci in necrotic lymph follicles in tonsils. Many large forms breaking into small forms. A few large and small diplococci in hemorrhagic mesenteric gland. No bacteria in liver, pancreas, myocardium or kidney	Pleomorphic streptococcus from ventricular fluid, cerebrum, medulla and cervical cord, and diphtheroid-like baclus from brain
	Large and small streptococcus from brain, pons, and cervical cord and gram-negative bacillus resembling colon bacillus from brain
A few diplococci in cord. No bacteria in myocardium	Medium sized streptococcus and large and small diplococcus from cerebrum, pons and cervical cord
Large and small diplococci in edematous capsule of spinal ganglia, in pia over anterior aspect of cord, in leukocyte in anterior horn, in pons, in edematous areas surrounding infiltrated blood vessels, in mesenteric gland and in contact films from cerebrum (Fig. 6). No bacteria in sections of brain, liver, heart muscle, or kidney. Diplococci in areas of infiltration in sections of cord after in glycerin for 15 months. Many organisms exactly the same in necrotic follicles of tonsils	Pleomorphic streptococcus from blood, spinal fluid, cerebrum, pons, cervical cord, and mesenteric gland. No bacteria in kidney, liver or spleen. Colon bacillus from lumbar cord and mesenteric lymph gland. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 15 months

TABLE 1—*Continued*

RESULTS OF A STUDY OF CASES OF ACUTE EPIDEMIC POLIOMYELITIS

No.	Age in Years	Duration in Days	Clinical Findings	Necropsy Time after Death	Microscopic Changes
734	7/12	6	Typical ascending paralysis	Day after death	Marked hemorrhages and diffuse infiltration in anterior horns of cervical cord. No changes in brain
745	5½	5	Paralysis of muscles of deglutition and left side of face. Death from respiratory failure	15 hours	Moderate perivascular and slight diffuse infiltration and numerous hemorrhages in gray matter of medulla and cervical cord. Hemorrhages in mesenteric gland. Small areas of necrosis in liver. No changes in lung, pancreas, myocardium and kidney
778	2	15	Paralysis of extremities, muscles of deglutition. Death—respiratory failure	2 hours	Moderate perivascular and circumscribed areas of round-cell infiltration in anterior horns and pia of cord. Marked degeneration and phagocytosis of ganglion cells
779	2	3	Brought to hospital in moribund condition. Death from respiratory failure	20 hours	Moderate perivascular and diffuse infiltration in gray matter of cord and pia. Many hemorrhages chiefly in anterior horns. Many of the infiltrating cells are polymorphonuclear leukocytes
938	12	3	Paralysis of muscles of deglutition, neck and arms. Death from respiratory failure	1½ hours	Extreme diffuse and perivascular infiltration in anterior horn of cord, in medulla and pons; slight in brain. Hemorrhage and leukocytes in alveoli of lung. Hemorrhage in mesenteric lymph gland. Small areas of round-cell infiltration in liver. Localized areas of leukocytic infiltration and hemorrhage in lung. No lesions of other viscera
943	2	1	Paralysis of left side of face, muscles of left leg and thorax. Death from respiratory failure	3 hours	Extreme round-cell infiltration, diffuse and perivascular in anterior horns of cord, moderate of pia and brain. Many of infiltrating cells are polymorphonuclear leukocytes. Extreme hyperemia and hemorrhage in mesenteric gland. Focal necrosis of liver with round-cell infiltration. Leukocytic infiltration in lung. No changes in myocardium, kidney or spleen
948	11	4	Paralysis of right side of face, muscles of neck and thorax. Death from respiratory failure	5 hours	Marked round-cell infiltration of gray matter of cord; perivascular infiltration and areas of hemorrhage. Areas of necrosis in lymph follicles of tonsil. Hemorrhage and hyperplasia of mesenteric lymph glands. Focal necrosis and focal round-cell accumulations in liver. No change in kidney or myocardium
949	17	3	Paralysis of legs, muscles of abdomen, back, upper extremities, of deglutition and diaphragm. Death from respiratory failure	½ hour	Extreme perivascular and diffuse infiltration with round cells in gray matter of medulla. Slight in pia and brain. Areas of hemorrhage and ganglion cell destruction. Areas of necrosis of lymph follicles in tonsils
960	16	3	Paralysis of muscles of deglutition. Death from respiratory failure	8 hours	Necrosis in lymphoid follicles of tonsils and adenoids. Hemorrhage of mucous membrane of stomach, perigastric and mesenteric lymph glands

TABLE 1—*Continued*

RESULTS OF A STUDY OF CASES OF ACUTE EPIDEMIC POLIOMYELITIS

Bacteria in Sections	Results in Cultures
A few diplococci in areas of hemorrhage in cervical cord. No bacteria in brain	Streptococcus and large diplococcus from brain and cord after in 50% glycerin for 15 months
Large and small diplococci in hemorrhagic areas of medulla. Small diplococci and chain of two in sections of anterior horns in smears from emulsions of cord (Fig. 7b) and in smears from cord after in glycerin for 15 months. Many similar diplococci in necrotic areas in lymph follicles of tonsils	Pleomorphic streptococcus from cerebrum, pons and cervical cord. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 2 and 15 months, respectively
A few large and small diplococci in pia and infiltrated areas in cord	
A few medium and exceedingly small diplococci in pia and in edematous areas surrounding perivascular infiltrated areas (Fig. 7a)	
Large and small diplococci in edematous areas around infiltrated blood vessels and infiltrated areas in medulla and lumbar cord, and in mesenteric gland. Very many similar organisms in necrotic areas in lymph follicles of tonsils. No bacteria in stomach, kidney, liver or spleen. A few diplococci in pneumonic areas in lung	Streptococcus and coccus forms from brain, pons, medulla, cervical and lumbar cord, and mesenteric gland. Colon bacillus from pons, lumbar cord, mesenteric gland. Kidney and liver sterile
Very large and medium coccus and diplococcus forms in medulla, pia over cerebrum. Many similar organisms in areas of necrosis in follicles of tonsils. A few in hemorrhagic areas in stomach. No bacteria in kidney, myocardium, pancreas, intestine or lung	Streptococcus and large coccus forms from brain, pons, lumbar cord and mesenteric lymph gland. Later also showed colon bacillus. Liver and spleen sterile. Large diplococcus in short chains and tetrads from brain and cord after in 50% glycerin for 3½ and 4½ months
A few diplococci in infiltrated area of anterior horn of cervical cord. Many similar organisms in tonsils. No bacteria in brain, myocardium, lungs, peribronchial lymph gland, mesenteric lymph gland or Peyer's patches	Pleomorphic streptococcus from brain. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 3½ and 4½ months
Diplococci and chain of two diplococci in pia and anterior horn and in smears from beneath dura of cord after preservation in glycerin (Fig. 7c). Large number of coccus forms singly in twos and short chains in lymph follicles of tonsils	Large and small coccus forms sometimes in short chains from brain, medulla and cervical cord. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 3½ and 4½ months
Enormous numbers of large medium sized and very small diplococci in lymphoid follicles of tonsils, adenoids and in lymph channels between muscle fibers outside of tonsil. Moderate number in hemorrhagic areas in mucous membrane of stomach and a few in mesenteric glands and lymphoid follicles of Peyer's patches	

"It appears to us that the small, filtrable organism which has been generally accepted as the cause of poliomyelitis may be the form which this streptococcus tends to take under anaerobic conditions in the central nervous system and in suitable culture mediums, while the larger and more typically streptococcic form, which investigators have considered contaminations, may be the identical organism grown larger under suitable conditions."

RESULT OF A STUDY OF CASES WHICH CAME TO NECROPSY

In Table 1 is given a summary of the clinical, pathologic, and bacteriologic findings of 19 cases of acute poliomyelitis in which necropsies were done.* The ages of the patients ranged from 7 months to 24 years; only 5 were more than 6 years. The duration of symptoms was from 1-15 days. The extent of flaccid paralysis varied greatly in the different cases. Nearly all these patients died of paralysis of the muscles of respiration. Bronchopneumonia was found in four; a terminal rise in temperature suggesting antemortem infection occurred in eight of the cases, but was absent in the others. The necropsies were all done within 24 hours after death; one in ½ hour, one in 1½ hours, two within 2 hours, and seven within 12 hours.

The gross and microscopic findings in all were typical of acute poliomyelitis. The viscera usually showed no gross changes other than congestion and cloudy swelling. The mesenteric lymph glands were swollen in most of the cases. Peyer's patches were swollen, and the lymphoid follicles in the intestines and mesenteric glands were edematous and hemorrhagic in cases in which there were marked gastro-intestinal symptoms. Localized hemorrhages in the mucous membrane of the stomach had also occurred in a number of the cases. As a routine, portions of tissues were immediately placed in 10% formalin and in some instances in Kaiserling's fluid, for microscopic study. They were embedded in paraffin, and sections from 5-10 microns thick were stained with hematoxylin and eosin, methylene-blue and eosin, and with eosin and polychrome methylene blue, for microscopic study.

The microscopic changes in the brain and cord were typical of poliomyelitis in all the 17 cases examined. The pia in all the cases showed some round-cell infiltration and in a few of the cases this was quite prominent, especially in the anterior fissure and over the anterior aspect of the cord (Figs. 1 and 3). Perivascular infiltration was variable but usually marked and consisted of small, round, deeply staining cells (Figs. 1 and 3). Infiltration was often very noticeable around the vessels which extend in horizontally from the pia in the anterior aspect of the cord (Fig. 3). Round-cell infiltration occurred in gray and white matter and in the ganglia, but was especially marked in the anterior horns. This was usually diffuse over wide areas, but small circumscribed areas were found adjacent to, and at times widely separated from, areas of diffuse infiltration. The cells were larger and stained less deeply than those around the blood vessels and often contained elongated nuclei. Neurophagocytosis was prominent in some of the cases. Only moderate infiltration was found in the posterior root ganglia. Polymorphonuclear cells were found in infiltrated areas in all but the 2 patients (Cases 714 and 778) who died 15 days after the onset of paralysis. In the others they were usually few in number, although in some running a short course they were quite numerous. In three cases fully one-third of the cells in some areas were polymorphonuclear leukocytes (Cases 779, 938, and 943). This finding confirms the observations made by Robertson and Blanton, namely, that polymorphonuclear cells appear to be especially prominent in the patients who die early, and are prone to be absent or few in number in those who die relatively late.

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* The material from nine cases was obtained through the courtesy of Drs. Anna Williams and H. L. Abramson of the Health Department of New York City.

Sections of mesenteric lymph glands, Peyer's patches, and, less often, of the spleen, showed hyperemia, hemorrhages, and, in some, hyperplasia of the lymph follicles. The liver in 4 cases showed the small areas of necrosis with the accumulation of round cells described by Peabody, Draper and Dochez. The lungs in 4 cases showed hemorrhage and leukocytic infiltration. In the rest of the organs there were no noteworthy changes aside from a certain degree of congestion of the vessels and granular swelling of parenchymatous cells.

THE DEMONSTRATION OF BACTERIA IN THE CENTRAL NERVOUS SYSTEM

Search for bacteria was made in the brain and cord and other organs in 14 cases, and in the brain and cord in 3 additional cases. The material studied consisted of films made directly from the brain surface, from material aspirated with pipets from the anterior horns and from emulsions in salt solution of brain and cord and mesenteric glands, and of sections of brain, cord, ganglia and other viscera. The latter were usually fixed in 10% formalin and in some instances in Zenker's or Kaiserling's solution. Sections were stained for bacteria by Gram-Weigert's or Giemsa's method, and with eosin and polychrome methylene blue. The films were usually stained by Giemsa's method. In the Gram-Weigert method decolorization was carried to the point where the cells showed a pale-blue tinge. If decolorization is carried farther than this the micro-organisms may lose the stain and the areas showing lesions cannot be made out clearly. Artefacts such as precipitated stain, cross-sections of nerve fibers and chromatin granules have been eliminated.

Coccus and diplococcus forms in chains of two or three and in small masses were found quite readily in sections of the central nervous system which showed typical poliomyelitic changes. They were demonstrated in all but 1 of the 17 cases. In the negative case, material from the brain only was available for study. Both film preparations and sections showed unmistakable organisms (Figs. 2, 4, 5, 6 and 7). They were found in contact films from the cerebrum, in films from the pipetted material from edematous anterior horns and in emulsions of the cord. The identical organisms were found in sections, in edematous areas in the dura, in the infiltrated pia, in the anterior fissure and surrounding the vessels in the pia along the anterior aspect of the cord, in edematous dura surrounding the intervertebral ganglia, in infiltrated ganglia, in areas of perivascular and diffuse infiltration in the gray matter of the cord, in walls of blood vessels and in dilated capillaries in edematous areas in the anterior horns, and in infiltrated areas in the brain. In 2 cases they were found in the cord, but not in the brain. These findings are in accord with those recently reported by Hektoen, Mathers and Jackson.

The same organism was found in small numbers in sections of mesenteric lymph glands in 6 cases. Diplococci of medium size were found in infiltrated areas in the lungs in 4 cases, and in hemorrhagic areas in the mucous membrane of the stomach, and in Peyer's patches in 2 cases.

No bacteria were demonstrable in sections of the other organs or in areas of brain and cord which were free from lesions. Bacilli or other microbic forms have not been found by us.

The micro-organisms were usually extracellular, but occasionally were found within cells. They varied greatly in size, shape and grouping in different locations in the same case, but were much alike in the different cases and occurred in widely separated regions. The variations in size were noted even among the minute forms and when arranged in short chains (Fig. 5b). The very small forms often occurred in groups commonly containing 4 or 8 and sometimes 16 minute cocci, usually in pairs, suggesting the rapid breaking up of large coccus forms into the small forms (Figs. 2c, 6b, and 7a). The very large coccus or diplococcus forms usually occurred along blood vessels or capillaries.

CULTURES FROM THE TISSUES

The cultures were made essentially according to the methods used by one of us in a study of the bacteriology of tissues from various diseases,[15] and that used by Flexner and Noguchi. To obtain the initial growth three mediums were regularly used: (1) Ascites-dextrose broth and dextrose broth in tall (12 cm.) and short (8 cm.) tubes and bottles with or without sterile tissue; (2) ascites-dextrose-agar and dextrose-agar, usually without tissue, and (3) tall tubes of unheated ascitic fluid plus sterile tissue and oil. The dextrose broth and agar were prepared with Witte's peptone and Liebig's extract of beef, titrated to 0.6-0.8 acid to phenolphthalein. The broth contained 0.2% dextrose, the agar 1% dextrose and 1.5% agar. The ascites fluid was of high specific gravity, was free from bile, and was previously proved sterile by making aerobic and anaerobic cultures from the sediment of lots of approximately 200 c c each. Pieces of brain, medulla, cervical cord, etc., were removed with sterile precautions and emulsified in a sterile air chamber or fragments planted directly. In some instances pipetted material from the anterior horns or the brain, after searing the surface, were also cultured. Usually a large number of tubes were inoculated with varying amounts of emulsions. Approximately 1 c c of a 10% emulsion in normal salt solution per 10 c.c. of medium gave the best results. Both too little and too much emulsion per cubic centimeter might lead to negative results. The cultures were incubated at 35 C. Only a few were put in the anaerobic jar, as it was thought desirable to make frequent smears of all cultures which grew.

Cultures were made from different parts of the central nervous system, usually of spinal or ventricular fluid, brain, pons, medulla, edematous dura, ganglia, and from different levels of the cord. A gram-staining coccus varying greatly in size, shape and grouping, but usually essentially streptococcal in character, has been isolated in all of 15 cases. In eight of the strains, chain formation was relatively marked and in seven it was relatively slight. In most instances the emulsions of all the specimens cultured yielded this organism, but not in all the tubes inoculated. The spinal fluid cultures remained sterile except in two instances, and these showed the characteristic streptococcus. Colon bacilli were obtained usually in only one or more fragments or tubes from 6 cases, hemolytic streptococcus in one instance, diphtheroid bacilli in two, and in a few instances Bacillus subtilis and Staphylococcus albus.

Cultures of emulsions of brain and cord after preservation in 50% glycerol in the ice-chest for from 3-16 months have yielded microorganisms identical to the one isolated from the fresh tissues in all but two of 16 sets of cultures which were made from material from 8 cases.

Cultures from mesenteric lymph glands were made in 4 cases. All yielded the characteristic micro-organism and in 2, colon bacilli were obtained in addition. The streptococcus isolated constantly from the central nervous system was not found in a single instance in other organs cultured in 5 cases. This result does not bear out the opinion expressed by Kolmer and Freeze, that this organism was probably present in these organs, but adds materially to the significance of their complement-fixation tests with antigens prepared from these strains.

The cultures in the short tubes of dextrose broth and bottles showed usually a diffuse granular turbidity in from 24-48 hours. Those inoculated with emulsions grew more often than those inoculated with fragments. Smears at this time showed cocci and diplococci often in short chains and occasionally in long chains of uniform size or, more often, of very irregular size. Subcultures on blood-agar plates at this time gave a growth of small, dry, green-producing colonies which might be surrounded by a narrow, hazy zone of hemolysis, especially after 48-72 hours' incubation. In old cultures in the short tubes of ascites-dextrose broth and, with few exceptions, in tall tubes containing this medium, the organisms become gram-negative as they die, but do not grow to small size (Figs. 12a and 13a). In shake cultures of the emulsion in short tubes of ascites-dextrose-agar small, discrete, grayish colonies, always in small numbers, appeared in the deeper portion of the medium, never above 1.5 cm. from the top, usually on the 2nd or 3rd day. The cultures in ascites fluid plus tissue and oil grew better when inoculated with fragments of nervous tissue than with emulsions. Growth in these tubes rarely became evident before the 3rd, more often after the 5th to the 10th day. Growth was manifested by the appearance of a diffuse cloud in the lower portion of the tubes which gradually increased and extended to the upper portion of the medium, but it rarely extended above about 3 cm. from the top. As this occurred the cloud in the lower portion became granular, a sediment appeared, and small colonies collected along the sides of the tubes. Tubes inoculated with emulsions showed similar but less constant results.

Smears from the bottom of the tubes of ascites-tissue fluid in which clouding was perceptible as early as the 3rd or 5th day, showed coccus and diplococcus forms as large as in the aerobic cultures together with numerous smaller forms. After a week or 10 days these cultures and those in which clouding first appeared in from 7-10 days showed enormous numbers of extremely small cocci, diplococci and short chains, often occurring in clumps. These small forms lost the Gram stain quite readily, stained purplish with Giemsa, were fully as small as, and appeared in every way to be identical with, the "globoid" organism described by Flexner and Noguchi. The organism isolated in ascites-dextrose broth remains an ordinary-sized diplococcus when grown aerobically. Transfers of aerobic cultures even after repeated platings to ascites fluid plus tissue and oil always resulted in growth of the small forms. Filtrates through Berkefeld N filters of these cultures have repeatedly yielded the organisms characteristic of these mediums. Cultures which showed only globoid bodies and which would not grow in aerobic broth or on plates, were grown back to large lanceolate diplococci and cocci without difficulty if they were not more than 3 or 4 weeks old. Those which had been under anaerobic conditions for a longer time could be grown back to large size, but with greater difficulty. Transfer of rather large amounts of the culture to short tubes of ascites-dextrose broth plus tissue was a good method to bring this about. Another good method to obtain the large aerobic forms was to stab tall tubes of ascites-dextrose-agar plus tissue and to make subcultures from the highest point of growth.

Generally speaking, there was a marked tendency of the small organisms in the anaerobic cultures to grow large when placed under aerobic conditions. It has frequently happened that shake-cultures from these in ascites-tissue-agar showed exclusively small organisms in colonies which grew only in the lower portions of the tube and which, on being transferred to ascites-dextrose broth showed large organisms varying greatly in size and shape, some resembling streptococci, others staphylococci and diphtheroid bacilli. These at first were considered contaminations, but since the different forms occurred in the same chain, and developed in strains after repeated platings, fishing single colonies each time, under conditions in which entrance of extraneous organisms was carefully excluded, they were held to be variants or mutations forms. Moreover, by following the methods of cultivation described, the details of which are to be published in another paper, two strains of pure cultures of the "globoid" organism obtained through the kindness of Dr. Noguchi have been made to grow aerobically on repeated occasions. This has been done with cultures made from single colonies and in media where the possibility of contamination from the tissue and ascites fluid was excluded. In the aerobic condition the size, morphology and character of growth resemble closely the aerobic form as we isolate it constantly from poliomyelitic tissue.

At first it was thought that the small globoid organism in ascites-tissue fluid (Fig. 13b), as described by Flexner and Noguchi, was characteristic of this medium, but by making smears from the bottom of the tubes daily after inoculation, it was found that the minute forms observed after some days of incubation and after evidence of growth was apparent, were the result of the breaking down of larger forms present before clouding occurred. Mathers, and Nuzum and Herzog have reported similar cultural results in cases of poliomyelitis, but appear to have overlooked this point. Kolmer, Brown and Freeze,[7] who also report similar findings, state that "Films of the aerobic and young anaerobic cultures showed a gram-positive coccus usually arranged in diplococcus formation in chains of four or five pairs and in clumps. Films of older anaerobic cultures (fourteen days or more) showed that the majority of these cocci had become smaller and many were easily decolorized by alcohol in the Gram stain."

A detailed study of this point was made by Rosenow and Towne with identical strains isolated from the brain and cord of monkeys paralyzed with virus in the usual way. By studying the method of growth in accordance with oxygen-tension the mechanism by which the large forms become small and the small forms large has been determined. They grow small in anaerobic cultures of ascites fluid plus tissue and oil and large in aerobic cultures of ascites-dextrose broth. Rosenow and Towne have already suggested that "among many factors concerned in causing these changes in size, surface tension may be of importance. If there are so many large diplococci that their metabolic requirements cannot be met by the medium, division which gives more surface for the same sized body might allow a certain number of the resulting smaller forms to survive."

The growth of the small organisms to large size under aerobic cultivation in ascites-dextrose broth where oxygen and available nutrient material are abundant would seem to be the result of the same factors working in the opposite direction. It appears quite as if the alterations in size occur according as certain metabolic or oxygen requirements need to be maintained. The occurrence of large, medium-sized and small forms in the tissues as shown in this paper would appear to be due to the same causes. The fact that large forms are prone to occur along blood vessels in the tissues where available oxygen would appear to be relatively high is thus explained, and hence these large forms should not be considered as being contaminations or as distinct from the small forms. A certain degree of adaptation or alteration in these requirements, however, appears to take place, because the organism, when kept under strict anaerobic conditions for a long time resists aerobic cultivation, a power which it can again be made to acquire gradually. The fact that unusually large forms are prone to develop when growth does occur (Figs. 13c and 13d) after anaerobic cultivation, and when first isolated from the tissues (Fig. 11), a property which is lost on continued aerobic cultivation, is also in harmony with this idea.

CHARACTERISTICS OF THE MICRO-ORGANISM FROM POLIOMYELITIS

In Table 2 is given a summary of the morphologic character, of growth on blood-agar, solubility in bile, etc., and the fermentative powers of strains isolated from tonsils and central nervous system of 35 cases of poliomyelitis. Altogether, the fermentative powers of strains from 49 cases have been studied, but the other tests were not made at the same time and hence are not included in the table. Twenty of the strains summarized in the table were isolated from the tonsils or the throat, 3 from stool and 17 from brain or cord. The culture generation is indicated by the figure following the point and

TABLE 2

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Strain	Source	Character of Growth on Blood-Agar Plate	Ascites Dextrose Broth		
			Turbidity	Sediment	Morphology
686.^{1}2	Tonsil	Small dry colonies with green halo. No hemolysis	Diffuse granular cloud	Granular	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
686.^{2}2	Tonsil	Small dry colonies with green halo. No hemolysis	Diffuse granular cloud	Granular	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
686.2	Tonsil	Small colonies with green halo. No hemolysis	Diffuse granular cloud	Flocculent	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
698.3	Tonsil	Grayish green indifferent colonies	Diffuse granular cloud	....	Irregular diplococci in short chains
699.7	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular diplococci in short chains
700.3	Tonsil	Small colonies with green halo	Diffuse cloud	....	Short chained pneumococcus-like streptococcus
701.4	Tonsil	Grayish indifferent colonies.....	Diffuse cloud	....	Short chained pneumococcus-like streptococcus
704.7	Tonsil	Moist colonies with green zone	Slight granular cloud	Flocculent	Irregular diplococci often in short chains
707.10	Cord	Small dry colonies with narrow green zone	Slight granular cloud	Flocculent	Irregular micrococcus type. Short chains and tetrads
707.4	Cord after in glycerin 16 months	Small dry colonies. Faint green zone in first two cultures then slightly hemolyzing zone	Diffuse granular cloud	Flocculent	Irregular short chained streptococcus
708.10	Tonsil	Medium dry colonies...........	Diffuse cloud	Flocculent	Medium size, decidedly irregular diplococcus in short chains
708.^{1}5	Tonsil	Small dry colonies..............	Diffuse cloud	Flocculent	Medium size, regular round diplococcus in short chains
711.3	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculetn	Pneumococcus-like diplococcus in short chains
714.11	Brain	Small dry colonies with green halo	Diffuse cloud	Granular	Round short chained streptococcus
714.^{1}8	Brain	Small dry colonies with green halo	Diffuse cloud	Granlar	Irregular streptococcus. Involution forms and cocci dividing transversely
714.^{2}7	Tonsil	Moist colonies with green halo	Diffuse cloud	Flocculent	Small round regular medium chained streptococcus
714.^{2}2	Brain	Moist colonies with green halo	....	....	....
714.^{4}7	Brain	Large moist colonies with green halo	Diffuse cloud	Flocculent	Round medium short chained streptococcus
714.3	Brain after in glycerin 16 months	Small dry colonies with green in first culture, slightly hemolyzing colonies in third culture	Diffuse cloud ular cloud	Flocculent	Round short chained streptococcus
721.5	Brain	Small dry colonies with green halo and larger more opaque grayish colonies	Diffuse cloud	Granular	Short chain streptococcus, varying size and shape, coccus forms and chains from single colonies
722.4	Brain	Small dry colonies with green green halo	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies
722.3.^{1}2	Brain	Translucent colonies	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies
722.^{1}2	Brain	Small colonies with green halo	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies

TABLE 2.—*Continued*

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Litmus Milk			Gelatin	Precipitation in Ascites Dextrose Agar	Solubility in Bile	Acid Production in Broth								
Acidity	Decolorization	Coagulation				Plain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin
+	0	0	0	+	0	0	+	+	+	+	No growth		..	0
+	0	0	..	..	0	0	+	+	+	+	+	No growth	+	0
+	0	+	..	..	..	0	+	+	+	+	+	No growth		0
+	0	0	0	..	..	0	+	+	+	+	+	0	+	+
+	0	0	0	+	0	0	+	+	+	0	0	0	+	0
..	..	..	..	..	..	0	+	+	+	+	+	No growth	+	+
..	..	..	..	..	..	0	+	+	+	+	+	0	+	+
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	0	0	0	+	..	0	+	+	+	+	+	0	+	0
+	+	+	0	+	0	0	+	+	0	+	+	0	0	+
+	0	+	0	0	..	0	+	+	+	+	+	0	0	0
No growth			0	0	+	0	+	+	+	+	0	0	0	0
+	0	+	0	0	0	0	+	+	+	+	0	0	0	+
+	0	+	0	+	0	0	+	+	+	+	+	0	0	0
+	+	+	0	0	0	0	+	+	+	+	+	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	+	+	+	+	0	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	+	+	Liquified	+	0	+	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	+	+	+	0
..	..	..	..	..	..	0	+	+	+	+	+	0	+	–
..	..	..	..	..	..	0	+	+	+	+	No growth		+	+
..	..	..	..	..	..	0	+	+	+	+	+	No growth	+	+

TABLE 2.—*Continued*

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Strain	Source	Character of Growth on Blood-Agar Plate	Ascites Dextrose Broth		
			Turbidity	Sediment	Morphology
723.5	Tonsil	Chocolate colored colonies......	Granular cloud	Granular	Irregular diplococcus ....
474.7	Cord	Moderate moist colonies with green halo	Slight cloud	Granular	Irregular short chained streptococcus hemolyzing pneumococcus
725.2	Tonsil	Chocolate colored colonies......	Slight cloud	Granular	Irregular short chained streptococcus resembling pneumococcus
729.10	Cord	Small moist and indifferent colonies	Diffuse cloud	Flocculent	Large oval diplococcus. No chains
729.7	Brain	Large moist indifferent colonies	Diffuse cloud	Flocculent	Medium round diplococcus........
730.10	Tonsil	Small dry green colonies........	Dffiuse cloud	Flocculent	Small round diplococci in clumps and short chains
731.2	Tonsil	Grayish colonies	....	....	Small round diplococci in clumps and short chains
732.8	Tonsil	Small dry colonies with green halo	Granular cloud	Flocculent	Very irregular streptococcus, short and long chains
733.2	Tonsil	Small dry colonies with green halo	Granular cloud	Flocculent	Very irregular streptococcus, short and long chains
734.2	Tonsil	Small dry colonies with green halo	....	....	Very irregular streptococcus, short and long chains
773.2	Stool	Moderate moist colonies with green halo	Diffuse	Slightly flocculent	Large micrococci singly in diplococci and short chains
836	Brain or cord	Small dry colonies with green halo	Diffuse	Granular	Large oval diplococcus..........
837	Brain or cord	Grayish green staphylococcus-like. Some hemolyzing colonies	Flocculent	Flocculent	Micrococcus type ....
838	Brain or cord	Grayish green staphylococcus-like. Some hemolyzing colonies	Flocculent	Flocculent	Micrococcus type ....
839	Brain or cord	Indifferent staphylococcus-like colonies	Diffuse	Granular	Micrococcus type. Quite staphylococcus-like
840	Brain or cord	Small dry colonies; slight hemolysis around colonies	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
841	Brain or cord	Small dry colonies; slight hemolysis around colonies	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
842	Brain or cord	Small dry colonies with green halo	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
899	Pons	Opaque grayish green colonies	Slightly diffuse cloud	Flocculent	Micrococcus type, tetracocci, diplococci and short chains
938.6	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular short chained streptococcus
938.7	Pons	Small dry colonies with green halo	Diffuse cloud	Granular	Irregular short chained streptococcus
943.6	Stool	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.6	Tonsil	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.3	Brain	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.3	Lumber cord	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
948.3	Tonsil	Large opaque colonies with greenish tinge	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size
948.3	Brain	Large opaque colonies with greenish tinge	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size
979.3	Stool	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size

TABLE 2.—*Continued*

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Litmus Milk			Gelatin	Precipitation in Ascites Dextrose Agar	Solubility in Bile	Acid Production in Broth								
Acidity	Decolorization	Coagulation				Plain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin
..	..	..	..	..	..	0	+	+	+	+	+	0	+	0
0	0	0	0	0	+	0	+	+	+	+	+	+	+	+
..	..	..	..	..	..	0	+	+	+	+	+	0	0	0
+	+	0	0	+	0	0	+	+	+	+	+	+	+	0
+	0	0	0	+	+	0	+	+	+	+	+	+	+	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	..	+	+	+	No growth		+	0
+	0	0	0	0	0	No growth	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	+	+	+	+	+	No growth		+
..	..	..	..	..	..	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	No growth	0	+	0
+	0	0	0	+	0	0	+	+	+	+	0	+	+	0
0	0	0	0	+	0	0	+	0	+	+	0	0	0	0
0	0	0	0	+	0	0	+	0	+	+	0	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	0	0	Liquified	+	0	0	+	+	+	+	0	+	+	0
+	0	+	0	0	0	0	+	+	+	+	+	0	0	No growth
+	..	..	0	..	..	0	+	+	+	+	0	0	+	+
..	..	..	0	..	..	0	+	+	+	+	+	0	+	+
..	..	..	0	..	..	0	+	+	+	+	+	0	+	+
..	..	..	0	..	..	No growth	+	+	+	+	+	0	No growth	+
..	..	..	0	..	..	0	+	+	+	+	+	No growth		+
+	0	0	0	..	..	0	+	+	+	+	+	+	+	+
+	0	0	0	..	..	0	+	+	+	+	+	No growth	+	+
+	0	0	0	..	..	0	+	+	+	+	+	0	+	+

the animal passage by the exponent to the right and above the figure indicating the strain or case number. Standard blood-agar made from Liebig's extract of beef and Witte's peptone to which about 0.3 c c per 5 c c of agar of defibrinated human blood was added, was used for the plates. Twenty-nine of these strains produced small, dry colonies with a green halo and sometimes slight hemolysis after from 48-72 hours; 7 produced somewhat more moist colonies with a greenish tinge resembling virulent pneumococci; 8 produced small grayish or chocolate-colored, indifferent colonies, and 7, larger, more opaque, grayish-white colonies resembling staphylococci. On this medium the organims were quite uniform in size and resembled pneumococci but were usually smaller and free from demonstrable capsule. The growth in ascites-dextrose broth or dextrose broth in columns about 7 cm. tall of the strains soon after isolation was relatively slow. Diffuse turbidity or a flocculent growth collecting along the side of the tube was usually noted in from 48-72 hours unless the inoculation was heavy, when marked growth occurred in 24 hours. After cultivation for a time the growth became more rapid. Most of the strains on isolation showed a preference for relative anaerobic conditions. It was the rule that growth in these tubes began at the bottom and was then forced slowly to the top as oxygen-tension was lowered and as the bacteria multiplied. Frequently the top 0.5 to 2 cm. were perfectly clear, especially in large amounts of medium in bottles, when the deeper layers showed dense growth. This clear layer was not observed in the tubes to which a thick layer of oil was added. The preference for relative anaerobic conditions was usually lost after a number of aerobic transplants, but it was preserved and at times was regained on anaerobic cultivation in ascites-tissue fluid.

The morphology in ascites-dextrose broth, as given in the table, varied considerably in the different strains and according to the time of and method of previous cultivation. In all but 7 strains the form was distinctly streptococcal, smears showing short chains of diplococci resembling pneumococci, a smaller number of medium-sized cocci in pairs of varying size and occasional large and very small diplococcus and coccus forms (Figs. 11, 12 and 13). Most of the strains had been grown aerobically for some time and had lost much of the tendency to grow with marked variations in shape and size. They approached ordinary-sized diplococci or short-chained streptococci.

Practically all the strains acidified, but only a small number decolorized or coagulated litmus milk. Only one strain liquefied gelatin.

Tweny of 27 strains produced clouding of ascites-dextrose agar; 3 of 26 were dissolved by bile. All produced acid in dextrose, maltose and saccharose and all but 2 in lactose. Twenty of 45 strains produced acid in raffinose, 11 of 39 in mannite, 28 of 44 in salicin, and 17 of 48 in inulin. In 3 cases the strains from tonsil and brain or cord, in 1 case from tonsil, stool, brain and cord, and in 5 cases the strain before and after from 1-4 animal passages showed practically identical fermentative powers. This was true in some instances in which a given strain was passed through different species of animals, and hence the strains isolated from the animals were not contaminants. The power to produce acid in inulin by these strains; as is true with pneumococci from lobar pneumonia, tends to be lost on prolonged cultivation, especially on blood-agar. Thus, 6 strains produced acid soon after isolation but not later in repeated tests. Of the total number of 141 cultures in which the fermentation tests were made, 69 were made soon after isolation, 33 fermenting inulin, while of 72 in which the tests were made from 3-15 months after isolation, only 7 fermented inulin. All the strains tested from the Davenport epidemic fermented inulin and all these tests were made soon after isolation. None of the Philadelphia strains* (Cases 836 to 899) fermented inulin. All the tests were made after the strains had passed through numerous cultures.

It is noteworthy that none of the 3 strains isolated from the stool in 3 cases fermented mannite, and hence they should not be regarded as Streptococcus fecalis. The morphology and character of growth resembled closely the strains isolated from the tonsil and nervous system. Moreover they had common agglutinative properties.† Gastro-intestinal symptoms were pronounced in each of these patients. The remaining 7 strains showed large diplococci singly, in masses and rarely in short chains. Mathers, and Nuzum and Herzog, and Kolmer, Brown and Freese also report the isolation of these 2 types of strains. The former consider them as the same organism, the latter as distinct. These strains produced larger and more opaque colonies than the streptococci. It has frequently happened that both these types have been isolated from the same case, both varieties have developed from widely separated single colonies and from pure cultures of the streptococcus after repeated platings. The diplococcus or micrococcus type of growth is particularly prone to develop from the streptococcus when pure cultures of the latter in old anaerobic cultures in ascites fluid plus tissue and oil or ascites-tissue-agar stabs are transferred to dextrose broth and then plated, or to blood-agar plates directly. Observations made in Case 721 are similar to many others that have been made:

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* We are indebted to Dr. B. Lucke and Dr. M. Solis-Cohen for these strains.

† For the agglutinating power of these strains and their infective power, reference is made to other papers in this series.

Sept. 5, 1916.—Smears from single colonies in ascites-dextrose-agar and dextrose-agar shake-cultures showed chains and pairs, some evidently streptococcus, others resembling staphylococci. Blood-agar plates showed pure culture of small, dry, green colonies. One of these colonies was stabbed and inoculated into the agar. Sept. 9.—Blood-agar plate from stab made from above colony in dextrose-agar again showed pure culture of small, dry, green colonies. Single colonies from blood-agar plate to blood-agar plate were now transferred daily for 6 days. The power to produce green colonies gradually diminished but otherwise pure, fine, dry colonies were obtained in each plating. A single colony was again plated and inoculated into a tall tube of dextrose broth. The plate-to-plate culture again showed fine dry colonies, while platings from the broth the following day yielded countless small colonies a little more moist than before, showing no green or hemolysis and among them larger, more opaque staphylococcus-like colonies with large diplococci and tetracocci. The latter resembled exactly those obtained with the streptococcus from dextrose-broth cultures of the emulsion of the brain and cord in this case, and until this observation was made were considered contaminations. A close distinction, therefore, between these two forms should not be made.

Case 695.—N. L., a boy, aged 4 years. (Patient of Dr. G. T. Joyce, Rochester, Minn.)

July 28, 1916.—The illness began with cough, headache, high fever and marked constipation. Flaccid paralysis of the right leg developed on the second day, of the right arm and left leg on the third, and marked weakness of the left arm and difficulty in breathing on the fourth. Much mucopurulent material was found in the nasopharynx. The throat was moderately red. The tonsils were small and appeared quite normal on the surface but pus was expressed. The spinal fluid was under pressure, and clear. The patient recovered with marked persistent residual paralysis. This child, together with his older brother, aged 6 years, had an attack of bronchitis with fever about 10 days previous to the onset of the paralytic attack. Both showed a recurrence of the bronchial infection, but the older brother recovered without developing paralysis.

Aug. 3.—Blood-agar plate of pus from tonsil showed enormous numbers of fine, dry colonies with a green halo, a few fine hemolyzing colonies, and a few larger, more opaque colonies.

Aug. 4.—Blood-agar plate of pus obtained from tonsil the second time showed a similar result. Ascites-dextrose-broth cultures from pus and from single colonies showed streptococci varying greatly in size and shape. In some instances very large budding forms were seen in chains containing typical diplococci. Large coccuslike forms in chains were seen to break into two and four smaller forms (Fig. 11). Cultures from the tonsil on three subsequent dates showed a gradual diminution in the number of fine, dry, hemolyzing colonies.

Case 707.—W. M., a boy, aged 4 years, was admitted to the New York Hospital Aug. 18, 1916. The disease began 6 days previously with high fever and vomiting, after which the child was better for 3 days. He grew nervous and restless and again vomited a number of times 3 days previous to admission to hospital, developing weakness and asymmetry of the left side of the face, and difficulty in swallowing. The patient was inert and stuporous, with rigidity of neck and back. The respirations were rapid and performed mostly with the accessory muscles. He cried out weakly and was unable to take food. A spinal puncture was made; the fluid was not under increased pressure and 15 c c were withdrawn. There were 40 cells per cubic millimeter.

Aug. 19.—The child died of respiratory failure. There was a rise of temperature to 103 F. immediately before death. This patient had had tonsils and adenoids removed several months previous to the attack of poliomyelitis. A partial necropsy was obtainable. There were edema and hemorrhages of the dura, marked congestion of the vessels of the pia of the cord associated with marked edema, a thin fibrinous film covering the pia, and a markedly increased amount of clear spinal fluid. Cross-sections of the cord showed extreme hyperemia of the vessels, hemorrhages and edema, especially of the gray matter of the anterior horns. The spinal ganglia appeared edematous. One and one-half centimeters of dorsal cord were removed in a sterile manner, emulsified in a sterile air chamber and cultures made immediately in dextrose agar, and ascites-dextrose agar, in ascites, tissue fluid covered with paraffin-oil, and in plain and dextrose broth with and without sterile ascites fluid.

Aug. 21.—Ascites-dextrose broth showed granular turbidity, and smears showed large numbers of large and small gram-positive and gram-negative diplococci and short chains. The larger organisms resembled pneumococci. Films from one colony 1.5 cm. from the top of the ascites-dextrose-agar shake culture showed large lanceolate-shaped and smaller, more rounded diplococci. Sometimes both varieties occurred in the same chain. No other colonies were detected in the shake cultures. Ascites-fluid culture showed no increase in turbidity. Ascites-dextrose-broth culture showed diffuse granular cloud and stained films, short-chained, irregular streptococcus.

Aug. 23.—Blood-agar plate cultures of each of these showed pure growth of fine, dry, nonadherent colonies of streptococci surrounded by a faint green zone. The blood-agar plates which had been inoculated with the single colony in dextrose-agar were used to inoculate ascites-dextrose broth, and tall tubes of ascites-plain-tissue broth and ascites-tissue fluid to which a layer of oil was added.

Aug. 24.—The dextrose-broth culture showed diffuse granular cloud with flocculent sediment and many gram-staining diplococci of irregular size. The ascites-plain-tissue broth and ascites-tissue fluid showed no turbidity.

Aug. 26.—The latter two cultures showed a dense collection of fine colonies in the bottom, appearing as a granular growth loosely adherent to the side of the tubes. These became gradually fewer in number and smaller in size up to 3.5 cm. from the top. Above this point the mediums were clear. Smears showed lanceolate diplococci, a great many round coccus-like bodies, occasionally in short chains, some as large as Staphylococcus albus, others much smaller.

Aug. 28.—Smears from the bottom of the ascites-tissue-fluid culture showed an occasional large diplococcus and chains of diplococci and a large number of very small cocci occurring in clumps, grouped, singly, in twos, and chains of three or four diplococci.

Sept. 15.—The ascites-tissue-fluid culture showed slight cloud throughout the medium. Smears showed a great many extremely small cocci singly, in masses, in twos, and short chains. No large forms were found. Films from a corresponding culture of ascites-dextrose broth showed disintegrating, chiefly gram-negative diplococci and short chains, but no minute forms. Subcultures were made.

Sept. 16.—Blood-agar plates from ascites-tissue fluid showed no growth, and from the ascites-dextrose broth a few green colonies. The subcultures from ascites-tissue fluid into ascites-tissue fluid showed no cloud, while those in ascites-dextrose broth showed a diffuse cloud in the lower one-third of the tube.

Sept. 25.—Smears of these cultures were made again, 10 days after inoculation. The films from ascites-dextrose broth cultures showed ordinary-sized diplococci and streptococci varying somewhat in size (Fig. 12a). The films from the ascites-tissue fluid showed enormous numbers of exceedingly small cocci, diplococci, and short chains together with a few large coccus and diplococcus forms, degeneration forms as described by Flexner and Noguchi (Fig. 12b). Blood-agar plate from culture in ascites-tissue fluid 26 days after inoculation showed a few very small green colonies and a few larger more opaque, grayish colonies. Films of the former showed diplococci resembling pneumococci, of the latter, large coccus and diplococcus forms. Subsequent subcultures from the ascites-tissue fluid on blood-agar were always negative but yielded growths in ascites-dextrose-tissue broth and ascites-tissue fluid, characteristic for these mediums. Exactly similar results were obtained with this strain from the single colony in ascites-dextrose agar after it was passed through 2 guinea-pigs, plated once and then inoculated into ascites-dextrose broth and ascites fluid, plus tissue and oil (Figs. 12c and d).

All shake cultures in dextrose-agar remained free from colonies in this case save the one used for study, and the cultures in liquid mediums showed streptococcus in pure culture. This colony was stabbed Aug. 21 directly into the agar culture and platings showed only typical streptococcus colonies. Sept. 22 this stab showed in smears streptococci and diphtheroid bacilli with all gradations between the two forms in the same chain. The diphtheroid forms were present all along the line of inoculation, but were most numerous in the deeper portions of the stab. Smears at this time from the tube of ascites-tissue fluid inoculated from the single colony showed extremely small streptococci and minute diphtheroid bacilli with all gradations between. Platings at this time on blood-agar showed two types of colonies, one the usual fine, dry colony with green halo, the other a small brownish-gray, indifferent colony. The former showed streptococci, the latter diphtheroid bacilli. Subcultures into broth of the former yielded streptococci, of the latter diphtheroid bacilli. Owing to the close resemblance of the cultures in ascites-tissue fluid to the "globoid" organism cultivated in this medium by Flexner and Noguchi and the filtrability of the virus of poliomyelitis, filtration experiments were done and Sept. 11, 1916, pure cultures of the streptococcus were found in ascites-dextrose broth of a filtrate of a culture in ascites tissue fluid containing small forms from Berkefeld N filter, while the cultures from a dense porcelain filter were negative. Cultures from the cord, after being preserved in 50% glycerin for 3 and 15 months, yielded pure cultures of the streptococcus.

Sections of the cord showed typical poliomyelitic findings, moderate round-cell infiltration of the pia, particularly over the anterior aspect of the cord and in the anterior fissure, marked dilatation of vessels, marked round-cell infiltration and degeneration of ganglion cells in anterior horns and moderate perivascular infiltration (Fig. 1). Search for bacteria in sections revealed gram-staining cocci and diplococci of various sizes in the pia and in the anterior horns of cervical cord and medulla (Fig. 2).

Case 712.—L. K., a girl, aged 4 years, was admitted to the New York Hospital Aug. 22, 1916. No accurate history was obtainable but it was learned that illness began 4 days before admission. On admission the patient was drowsy, scarcely able to speak or breathe, and was unable to lift her head. There was weakness of the right shoulder and neck, flaccid paralysis of both lower extremities with the exception of slight flexion of the toes of the left foot, and absence of abdominal and knee reflexes. The spinal fluid was found to be under moderate pressure, slightly turbid, and 40 c c were withdrawn; there were 350 cells per cubic millimeter. Paralysis of the respiratory muscles developed and progressed rapidly, and death occurred Aug. 23.

At necropsy there was found edema surrounding the dura, especially in the cervical region, extending along the nerve roots and ganglia. The ganglia appeared edematous. The spinal fluid was clear and increased in amount. There was marked hyperemia of the vessels in the pia. There were hemorrhages and edema in the gray matter of the cord, especially in the anterior horns. The brain could not be obtained. The peritoneal cavity contained a small amount of clear fluid. The stomach contained a moderate amount of chocolate-colored fluid free from food. The mucous membrane was studded with numerous bleeding points, especially in the fundus, some of which showed superficial ulceration. The spleen was firm; the cut surface was moist. The lymph follicles were prominent as round, opalescent, grayish points. Kidneys, liver, adrenals, pancreas, pelvic organs, intestines, appendix and thymus showed no noteworthy changes. The tonsils were of normal size. Numerous cross-sections made with a razor, beginning at the base, showed a small abscess in each tonsil containing a peculiar semiliquid, opalescent pus. Cultures were made from the liver, spleen, kidney, spinal cord, and pus from an abscess in the tonsil. Those of the liver, spleen and kidney were sterile. Cultures of emulsions of the cord showed gram-positive cocci of varying size singly, in twos, and short chains. Blood-agar plates from cultures of cord showed two types of colonies, rather large grayish-white colonies resembling Streptococcus albus, but less opaque, and numerous smaller colonies resembling Streptococcus viridans. Ascites-dextrose-broth culture of tonsil pus showed short-chained streptococcus and large cocci singly, in pairs, clumps and short chains. Blood-agar plate of tonsil pus showed chiefly grayish-white colonies resembling staphylococcus colonies and nonhemolyzing colonies of streptococci.

Sections of the cord showed extreme perivascular and diffuse infiltration, chiefly of gray matter of the cord, medulla and pons. The perivascular infiltration was especially prominent around the vessels extending in from the surface of the cord. There was moderate infiltration of the pia, especially anteriorly, and in the anterior fissure, and marked degeneration of ganglion cells (Fig. 3). Sections of the viscera showed no changes other than hemorrhages in the mucous membrane of the stomach, and small areas of necrosis with aggregations of round cells in the liver. No bacteria could be detected. Fully 40 gram-staining diplococci or cocci of various sizes were found in the anterior horns of the cord, chiefly at the peripheral zone of circumscribed areas of cellular infiltration, in perivascular areas of infiltration in the pia and in the edematous dura, and in infiltrated ganglia and hemorrhagic areas surrounding the ganglia.

Sections of the tonsils showed a number of areas of necrosis in the center of the lymphoid follicle near the base of the tonsil, and a small amount of exudate in the crypts. The cells in the necrotic areas were made up chiefly of large and small mononuclear cells, but polymorphonuclear leukocytes were also present. The cryptic exudate contained many leukocytes and large mononuclear cells and debris. In the areas of necrosis numerous gram-staining cocci were found which varied greatly in size. Some were very large, singly or in twos, while others were exceedingly small, singly, in twos and in short chains. Large forms were found which showed all grades of division. Some were perfectly round, others only slightly elongated, some were breaking into 2 and each of these again dividing into smaller diplococci. Other round forms appeared to be breaking into 4 cocci, each of which in turn showed beginning division. Others were breaking into 8 small cocci, arranged in pairs, while still others were dividing into 8 diplococci and each of these showed a light line of beginning division too small to be photographed or drawn. Chain formation was slight but evident in some fields and appeared to be the result of transverse division of larger coccus forms in some instances. Both Gram and eosin, and polychrome methylene blue stains gave this picture (Fig. 10). Bacilli were not found in these areas. The exudate in the crypts was composed of necrotic cells and showed numerous gram-staining diplococci and short chains, large coccus forms and a moderate number of fusiform bacilli. Relatively few bacteria were found in parts of the tonsils showing no lesions, although cocci and diplococci of varying size, but always smaller and more streptococcal in shape and size, were found in perivascular areas of infiltration even beyond the capsule, along sheaths of muscle fibers, within capillaries, and in blood vessel walls.

CASE 714.—J. B., a girl, 2 years of age, was admitted to the New York Hospital, Aug. 13, 1916. Four days before admission she had fever, diarrhea, drowsiness and loss of appetite. On the 3rd day marked weakness of both legs appeared. On admission there was complete flaccid paralysis of both legs and left arm, with absence of abdominal and knee reflexes.

Aug. 22, the respiration became difficult and irregular. The following day respirations were wholly diaphragmatic, the temperature became higher, and edema of the lung and bronchopneumonia became evident. Death occurred Aug. 24.

The spinal fluid on admission was clear; 20 c c were withdrawn and 30 cells per cubic millimeter were found. Aug. 22, 14 c c of clear fluid were withdrawn and 4 cells were found.

At necropsy edema was found surrounding the dura, nerve roots and ganglia, especially along the cervical and lumbar enlargements. The ganglia appeared swollen and edematous. The cerebrospinal fluid was clear but increased in amount. Cross-sections of the cord showed marked edema and softening and small hemorrhages in areas in the anterior horns. The pial vessels over brain and cord showed extreme congestion. The pia arachnoid was edematous and showed a number of hemorrhages over the anterior aspect of the medulla. The peritoneal and pericardial cavities contained a moderate amount of clear fluid. There was moderate cloudy swelling of liver, kidney, and myocardium. The spleen was firm, the lymph follicles were prominent and the cut surface moist and bloody. The right lung was firm, mottled grayish-red and free from crepitation, with the exception of the anterior portion of the upper and middle lobes. The other viscera were normal. The tonsils were slightly enlarged but the surface appeared normal. Numerous cross-sections, beginning at the base, showed not less than 6 abscesses situated near the base, varying in size from 1-3 mm. All contained thick, opalescent, gray-yellow pus. The crypts contained a few small, firm plugs. Cultures of the tonsils and pus from the abscesses in the tonsil showed enormous numbers of fine, dry colonies of streptococci surrounded by a green zone and a few larger, more opaque colonies resembling Staphylococcus albus. No hemolytid streptococci were found. Cultures from the lung showed pure growth of diplococcus resembling pneumococcus. Those from the cerebral fluid, the edematous fluid surrounding the dura of the cervical cord, the cervical cord and the brain showed pure growths of green-producing, short-chained streptococcus varying greatly in size and shape. Cultures from the kidney were sterile. Those from the blood showed diphtheroid bacilli.

Numerous parallel cultures were made with the streptococcus isolated from this case in ascites-dextrose broth with and without tissue, and in ascites-tissue fluid plus oil. The character of growth in these mediums was studied also after the strains were plated and single colonies used for inoculation after they were passed through from 1-4 animals and after the brain of the animals and cultures containing the small forms were filtered. In all instances, if growth occurred, it was characteristic of these mediums, independently of the age of the culture (Fig. 13a). In the former it resembled streptococci or pneumococci with some irregularity in size and grouping. In young cultures there was usually a slight unstained zone surrounding the diplococci suggesting a capsule, while in the ascites tissue fluid the organism always became small in size and tended to lose the stain in Gram's method (Fig. 13b).

Sections of brain and cord showed typical changes of poliomyelitis, dilatation of vessels and hemorrhages in anterior horns, marked degeneration and slight phagocytosis of ganglion cells, moderate perivascular and diffuse infiltration in the gray matter of the cord, slight in brain pia and spinal ganglia. Sections of the tonsils showed hyperplasia of lymph follicles but no areas of necrosis. A moderate number of diplococci and cocci of various sizes were found in the lymph follicles and many in the crypts together with fusiform bacilli. Sections of viscera showed no noteworthy changes with the exception of the lung where hemorrhage and leukocytic infiltration were marked. Search for bacteria showed large and small cocci and diplococci in smears from the anterior horn and surface of cerebrum, and in sections of cord and intervertebral ganglia (Fig. 5). No bacteria were found in sections of kidney, spleen, myocardium and pancreas.

THE TONSILS AND ADENOIDS

The affinity of the virus of poliomyelitis for lymphoid tissue has been emphasized and so-called virus has been demonstrated in the tonsils. Notwithstanding these facts, however, little attention has been given to a study of the tonsils and adenoid tissue to determine the presence or absence of lesions, the number and kind of bacteria in the tissues, and the importance of these structures as affording entrance for the infective agent in poliomyelitis. At the very outset of this study, one of us was impressed by the fact that a relatively large amount of infective material could be expressed from tonsils which, on inspection, appeared quite normal. Peabody, Draper and Dochez found the tonsils and lymph glands enlarged in a high percentage of cases in their series, but appear not to have studied the tonsils after death. Microscopic examination of the material expressed and cultures made by us revealed the presence of cocci and streptococci which showed marked variations in size and shape (Fig. 11). Deep cultures in ascites-dextrose broth of the very first case studied (July 21, 1916) showed[21] "apparently pure cultures of streptococci in short and medium chains and in pairs. Some of the diplococci were large and lanceolate in shape, resembling pneumococci, and others were small and more coccus-like. Both large and small forms were found in the same chain."

Young rabbits and guinea-pigs (animals considered resistant to poliomyelitic virus) injected intravenously with freshly isolated cultures, developed flaccid paralysis.[20] These findings were strikingly different than had been obtained in a study of tonsils in other diseases and suggested the possibility that the "globoid" organism described by Flexner and Noguchi as the cause of poliomyelitis might be a form of streptococcus, and hence a detailed study of this question was undertaken.

Blood-agar plate cultures of the pus expressed from tonsils at the beginning of the attack as illustrated in the protocols, showed almost constantly large numbers of fine, dry, green-producing colonies of streptococci and a small but variable number of larger, more opaque, glistening colonies resembling staphylococci; usually a few small, slightly hemolyzing colonies of streptococci, a moderate number of Micrococcus catarrhalis and occasionally a few colonies of colon bacilli and other extraneous organisms. Typical hemolytic streptococci were almost never found except in cases which showed a complicating follicular tonsillitis. Cultures from abscesses of tonsils removed before and after death showed with remarkable constancy this same type of flora and frequently showed only the two first types of colonies. Cultures from single fine, dry, green colonies in ascites-dextrose broth often showed remarkable variations in size and shape (Fig. 11), and if again plated, frequently showed the fine, dry, green and larger, more opaque colonies, especially from old cultures.

In 17 cases, the patients ranging in age from 7 months to 24 years, in which the tonsils were examined after death by making numerous cross-sections with a razor, from 1-15 abscesses were found. These were usually situated near the capsule and were not connected with the crypts. They varied in size from 1 or 2 mm. to 5 mm. in diameter and usually contained a thick opalescent grayish-yellow material, softer and more opaque than the collections of cells from swollen follicles. Smears showed large and small mononuclear cells, leukocytes, debris, and later enormous numbers of diplococci of medium size, large and small coccus and diplococcus forms, and occasionally fusiform bacilli. The small forms resembled "globoids," but no sharp line could be drawn between these and the larger forms; all types were often seen in the same chain. On the basis of these striking findings, and after it had become evident that the organisms were of low virulence, it was determined through the cooperation of Dr. J. C. Roper to remove the tonsils in a series of patients who were not convalescing satisfactorily and in whom a low grade of fever persisted. This was done in 11 cases. In 3 patients there was a slight temporary rise in temperature, in 5 the temperature curve was not altered and in 3 it was definitely lowered. Seven of the patients showed improvement in their general condition, they were brighter and less irritable. In the others no noticeable change occurred.

During tonsillectomy a large amount of infected material was noted, and in all, one or more small abscesses were found in the tonsils on making sections. These abscesses were less prominent than in the fatal cases. Similar abscesses and identical organisms were found in adenoids removed before and after death. Sections of the tonsils showed marked hyperplasia of the lymph follicles and in all but 2 of the 14 cases studied, one or more small or large areas of necrosis in lymph follicles (Table 1 and Fig. 8). The exudate in the crypts contained leukocytes, round cells and many cocci and diplococci and moderate numbers of fusiform and other bacilli. In a number of instances the epithelial lining of the crypts showed atrophy but usually this could not be made out.

The cells in the necrotic areas in the follicles were chiefly large and small mononuclears, although in some instances a large number of leukocytes were present. Many large and small cocci and diplococci exactly like those found in small numbers in the brain and cord and mesenteric glands were found in these areas. Bacilli were almost wholly absent. Excellent opportunity to study the relationship between large and small forms was here afforded and in a number of instances unmistakable evidence of large coccus and diplococcus forms breaking into the smaller cocci were discovered (Figs. 9 and 10). The number of bacteria demonstrable within swollen follicles was surprisingly small. Those found corresponded very closely in size and shape to the cocci found in the cord and mesenteric lymph glands. Bacilli were never found in swollen but otherwise normal follicles. In a number of instances a large number of cocci and diplococci were found in areas of round-cell infiltration, in the capsule of the tonsil, and around large blood vessels outside the capsule. A few were found in blood vessel walls and within the lumen of blood vessels.

In the Davenport epidemic a study of the relation of infection in tonsils to enlargement of cervical lymph glands immediately outside the tonsils was made in 38 cases. In 26 of these the cervical glands were enlarged, and in 11 the enlargement on either side was in proportion to the amount of infection in the tonsil on the corresponding side, as determined by the greater size of the tonsil or larger amount of infected material which could be expressed. In several instances these findings were verified at necropsy. No definite relation between infected conditions of the teeth could be made out although caries was present in some of the cases. The tonsils from 12 persons removed for various reasons in other diseases have been examined in the same way. The abscesses in the lymph follicles on cross section and the areas of necrosis noted microscopically in poliomyelitis were not found, although abscesses in crypts containing many organisms with atrophy of the epithelium were found in some of these.

The results of the study of the tonsils and adenoids here reported indicate strongly that these structures afford important entrance ways for the micro-organisms which we find constantly in the diseased tissues in poliomyelitis. A relatively large amount of infected material may be expressed from the tonsils. The abscesses and areas of necrosis are more numerous and are larger in the fatal cases than in tonsils removed from convalescent patients. These abscesses contain large numbers of this micro-organism, particularly in the fatal cases, and the cocci have been demonstrated in tissues immediately outside the tonsils, in areas of infiltration along large blood vessels and within the lumen of capillaries. Injection of cultures into animals produced flaccid paralysis. Removal of tonsils and adenoids in convalescent patients has been followed by seemingly good effects in some instances. In view of these facts there can be little doubt but that diseased tonsils and adenoids predispose to poliomyelitis and may lead in some instances to severe or fatal infection which might otherwise be mild. The focal areas of infection with abscess formation in these structures may be an important factor in prolonging an attack, in preventing early restoration of lost muscle function and be the cause of remissions or relapses which occur not infrequently as pointed out especially by Wickman and by Draper. The abscesses are usually small, and so situated mechanically as not to interfere with their healing. Protection by removal of these structures is only relative at best and temporary entrance channels for bacteria are made by their removal. Hence, their wholesale extirpation for protection against this dread disease would not be justified. Their removal in special instances may be useful, but the well established principles for the removal of tonsils and adenoids as applied in connection with other conditions should be followed in this disease.

SUMMARY

A pleomorphic coccus has been isolated from and demonstrated in affected tissues in all the cases examined of poliomyelitis that occurred in different epidemics and in widely separated parts of the country. The organism has been found in large numbers in adenoids and tonsils, in smaller numbers in lesions in various parts of the central nervous system, and in the mesenteric lymph glands. It has been proved to be absent in organs showing no lesions. The organism shows great variations in size and shape, depending on the method of cultivation. In aerobic cultures, although more irregular in size, it closely resembles ordinary green-producing streptococci or pneumococci of low virulence. Under anaerobic cultivation, especially in tall columns of ascites fluid plus tissue and oil, it grows to very small size, becomes filtrable and in every way appears identical with the "globoid" organism described by Flexner and Noguchi. The variations noted in cultures have been proved not to be due to contaminations or to mixed cultures. All gradations in size between large coccus or diplococcus forms to exceedingly small, almost ultramicroscopic forms, were found alike in cultures and in the tissues, and the conditions favorable for the formation of one from the other have been determined. Morphologic evidence of the breaking down of large forms into the small forms in the tissues has been obtained. Pure cultures of this organism have been isolated many times and it has been demonstrated in films and sections of brain and cord many months after they were placed in 50% glycerol. In view of these results the presence of this organism in the diseased tissues cannot be considered an accidental contamination.

The following facts, determined since the studies reported in this paper were begun, indicate that the organism here described bears etiologic relationship to poliomyelitis. It is constantly present in the diseased tissues, from which it can be cultivated even many months after glycerolation. On injections of cultures into young rabbits and guinea-pigs it localizes specifically in the nervous system and produces flaccid paralysis and changes in brain and cord which resemble those in poliomyelitis in man.[20] From the brain and cord of these animals the organism can be isolated and the disease again produced. The organism has been rendered filtrable. By means of the same methods the identical organism has been isolated constantly from the brain and cord of monkeys paralyzed with fresh, glycerolated and filtered virus.[19] The serums of persons and of monkeys, having recovered from poliomyelitis, agglutinate specifically the more sensitive strains both from human and monkey poliomyelitis.[18] Injections of the recently isolated aerobic cultures into monkeys renders them refractory to virus. The aerobic form of the organism from human and monkey poliomyelitis produces antibodies in the serum of horses, in a large amount common for both, cross-agglutinating these strains specifically in high dilution.[19] The serum of a horse immunized with freshly isolated strains from monkeys protected monkeys relatively against intracerebral inoculation of virus[16] and had pronounced curative effects in the treatment of human poliomyelitis. Early intravenous injections were followed by almost immediate cessation of symptoms in a large series of cases.[17]

The results of Flexner and Noguchi, so far as the cultivation of a small filtrable organism and its demonstration in the tissues in poliomyelitis are concerned, have been corroborated, but the results of our experiments indicate that this is the anaerobic and, according to Amoss' results, a nonantigenic form of the organism which, under aerobic cultivation, clearly belongs to the streptococcus group of microorganisms. Both forms have been constantly demonstrated side by side in the tissues of poliomyelitis. Flaccid paralysis coming on soon after injection has been produced in monkeys with characteristic, although not typical changes in the cord with aerobic cultures, but the classic picture as obtained with virus in this species has not been secured . It may be suggested, however, on the basis of results already obtained, that this is due to the development of antibodies, since the organism in the aerobic form has marked antigenic powers.

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EXPLANATION OF PLATES 6-8

Fig. 1.—Section of upper segment of cervical cord (Case 707) showing typical poliomyelitic lesions. For explanation of letters see Fig. 2. Hematoxylin and eosin. × 20.

Fig. 2.—Micro-organisms in section of cervical cord shown in Fig. 1. Gram-Weigert. × 1000. Large and small diplococcus in edematous pia at *a*. Medium-sized diplococcus in pia at *b*. Small diplococci and cocci in anterior horn adjacent to blood vessel at *c*.

Fig. 3.—Section of cervical cord (Case 712) showing typical poliomyelitic lesions. For explanation of letters see Fig. 4. Hematoxylin and eosin. × 50.

Fig. 4.—Micro-organisms in cervical cord (Case 712) shown in Fig. 3. Gram-Weigert. × 1000. a. Large diplococcus adjacent to blood vessel at *a*. b. Medium small diplococcus, round cells and leukocytes at periphery of area of infiltration at *b*. c. Medium-sized diplococci in pia. d. Medium-sized diplococcus in ganglion at *d*.

Fig. 5.—Micro-organisms in nervous system of Case 714. × 1000. (a) Large and small elongated cocci in film from anterior horn. Giemsa. (b) One large coccus, medium-sized diplococci and very small diplococci in contact film from surface of cerebrum. Giemsa. (c) Large and small diplococci in edematous infiltrated area in anterior horn of cervical cord. Gram-Weigert. (d) Diplococcus in intervertebral ganglion. Gram-Weigert.

Fig. 6.—Micro-organisms in nervous system, Case 729. × 1000. (a) Diplococcus in contact film of cerebrum. Giemsa. (b) Large coccus form breaking into small diplococci and cocci, and a small lightly stained diplococcus in contact film of cerebrum. Giemsa. (c) Two medium-sized diplococci in chain and small diplococcus in pia near anterior fissure. Gram-Weigert. (d) Three diplococci in pia anterior fissure. Gram-Weigert. (c) Diplococcus adjacent to leukocyte in edematous area in anterior horn of lumbar cord. Gram-Weigert.

Fig. 7.—Micro-organisms in nervous system in cases of poliomyelitis. × 1000. (a) Group of small, lightly stained diplococci in infiltrated area surrounding blood vessel, Case 779. Gram-Weigert. (b) Diplococci in normal salt solution emulsion of cervical cord, Case 745. Gram-Weigert. (c) Chain of three diplococci in smear from beneath dura of cord of Case 949 after preservation in glycerol for 4 months. Giemsa.

Fig. 8.—Necrosis of lymph-follicle near base of tonsil, Case 712. Hematoxylin and eosin. × 40.

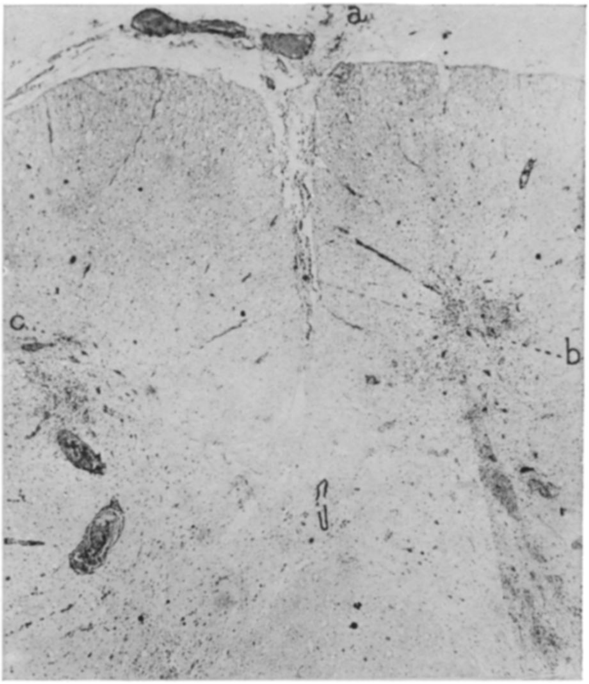
Fig. 9.—Large coccus and medium-sized diplococcus at *a* (Fig. 8), the former breaking into four small diplococci. Gram-Weigert. × 1000.

Fig. 10.—Composite drawing of various levels of field shown in Fig. 9, to show details of relation between large and small forms of micro-organisms and the mechanism of formation of the small forms.

Fig. 11.—Smears from 18-hour cultures in tall tubes of ascites-dextrose broth inoculated with single colonies of green-producing streptococcus from blood-agar plate inoculated with pus from tonsil, Case 695. Note the extreme variations in size, shape and grouping, and various stages of division of cocci in chain in *c*. Lanceolate diplococci undergoing fission into flat-sided diplococci and further longitudinal fission resulting in small forms. Gram. × 1000.

Fig. 12.—Smears from aerobic and anaerobic cultures of streptococcus isolated from the cord of Case 707. Gram-Weigert. × 1000. (a) Twelve-day culture in ascites-dextrose broth in third culture and derived from single colony in culture from cord, showing streptococcal type of growth. (b) Same as *a* but from tall tube of ascites-fluid plus tissue and oil, showing very small cocci, diplococci and short chains. (c) Same strain after two animal passages and from single colony on blood-agar plate. Forty-eight hour culture in tall tube of ascites-dextrose broth showing typical streptococcal type of growth. (d) Same as *c* but in ascites fluid plus tissue and oil after incubation for 24 days, showing extremely small cocci, diplococci and short chains and one large "degeneration" form.

Fig. 13.—Smears from cultures of streptococcus isolated from the brain in Case 714. Gram. × 1000. (a) Six-day culture in ascites-dextrose-tissue broth from brain of monkey paralyzed with culture in the fourth animal passage. Note quite typical streptococcal forms. (b) Same as *a* but culture in tall tube of ascites-fluid plus tissue and oil. Note small micrococcus and diplococcus type of growth. (c and d.) Eighteen-hour culture in ascites-dextrose broth of above strain after one plating and after five successive anaerobic cultures in ascites-tissue fluid. Note the extreme variations in size and shape and the yeast-like bodies occurring singly and in chains of typical streptococci.



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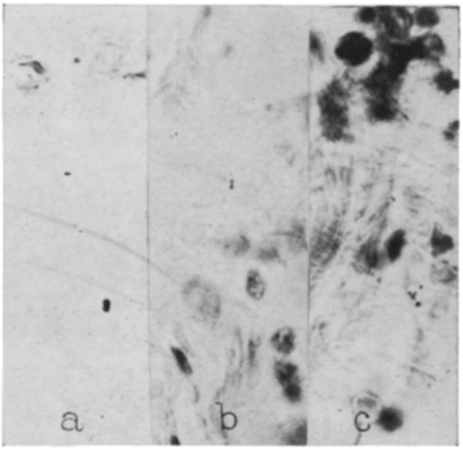

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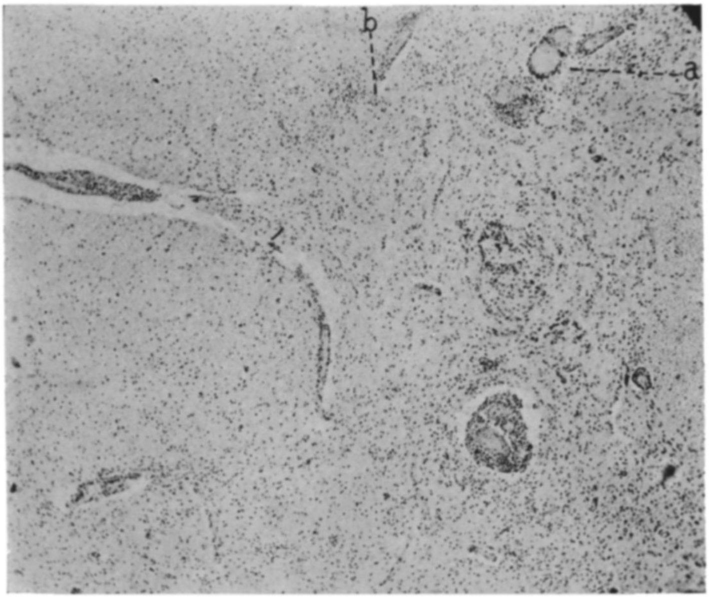

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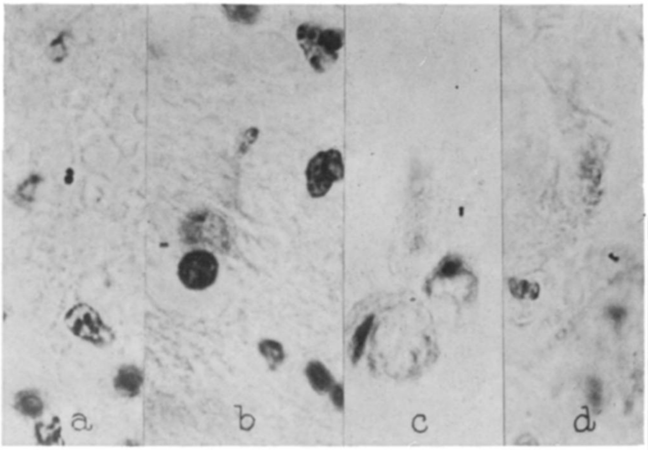
Plate 7



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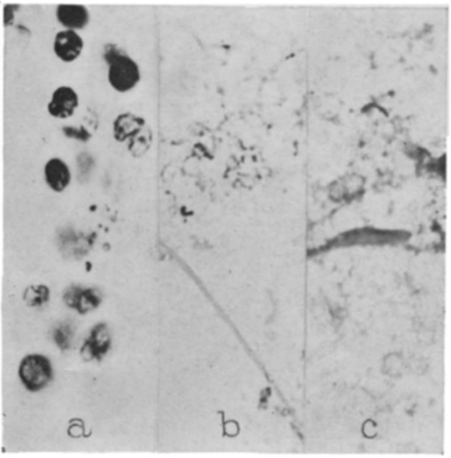

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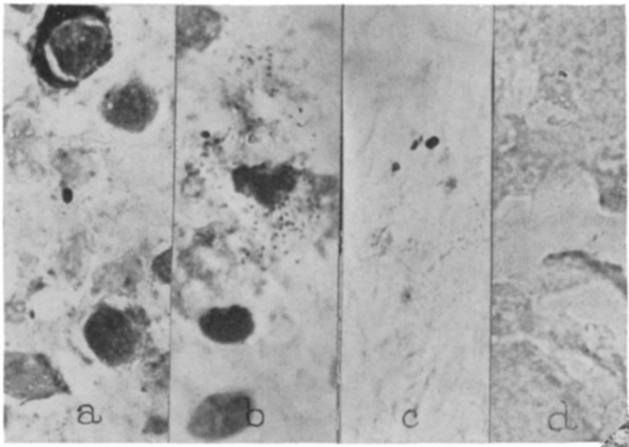

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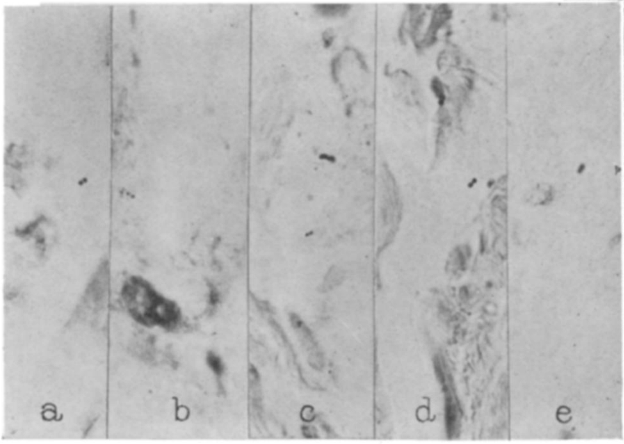

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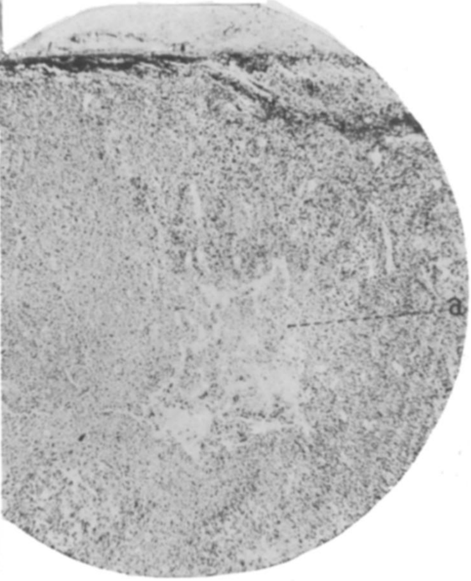
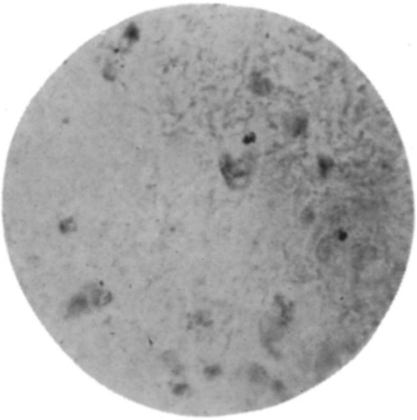
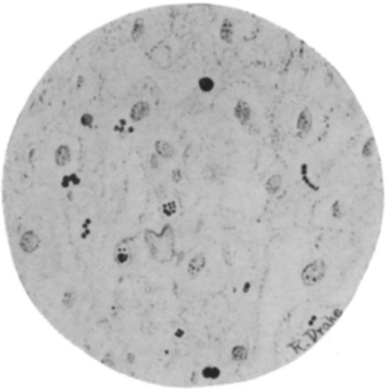


8

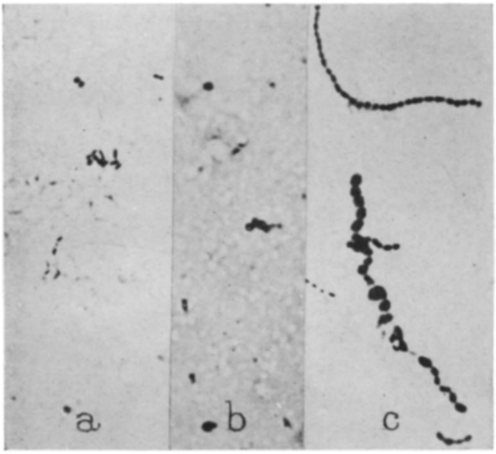
PLATE 8



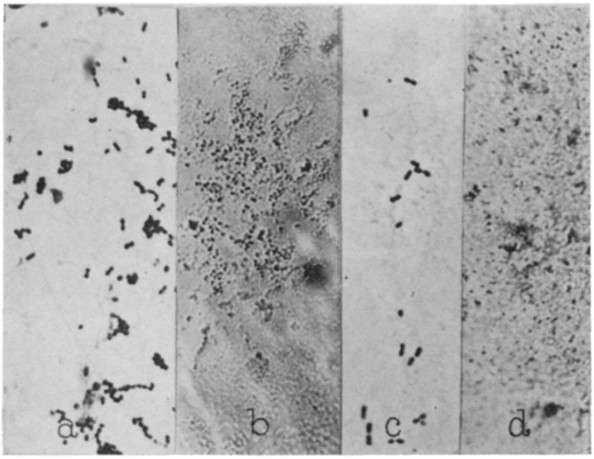
9



10



11



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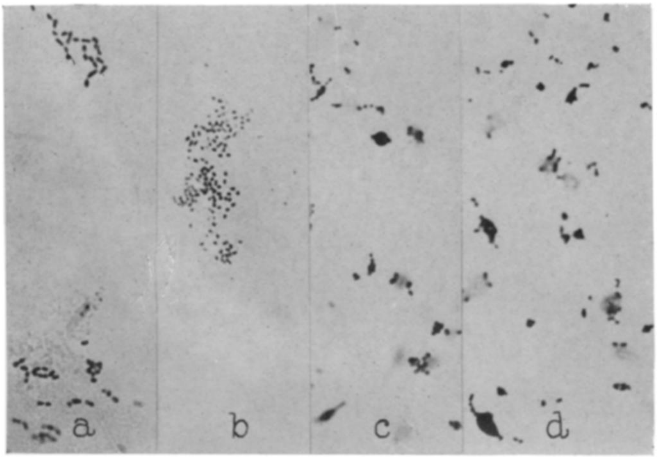

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